Integrating Information from Multiple Toxicity Testing Approaches in Cancer Hazard Identification

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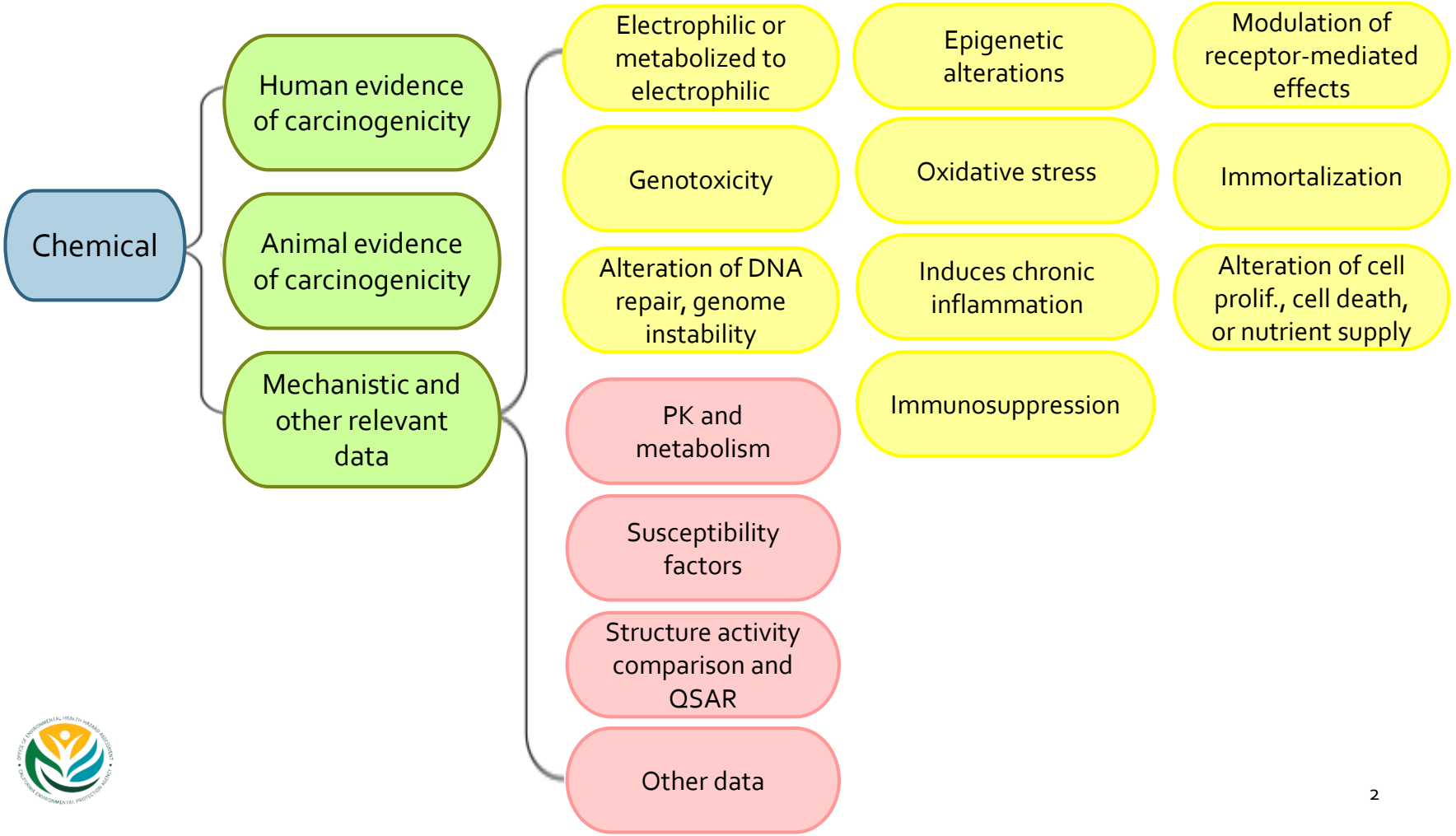
Integration of Evidence Streams in Cancer Hazard Identification



Sources of Mechanistic and Other Relevant Data

- "Traditional"
  - Studies in humans (pre-neoplastic changes, evidence for one or more of the key characteristics of carcinogens (KCs), genetic susceptibility, pharmacokinetics and metabolism)
  - Studies in animals (short-term models of carcinogenesis, including transgenic animals; pre-neoplastic changes; evidence for one or more of the KCs; genetic susceptibility; pharmacokinetics and metabolism)
  - Bacteria, yeast, and in vitro systems (tissue, cellular, and molecular-based) e.g., evidence for one or more of the KCs; genetic susceptibility; pharmacokinetics and metabolism
  - Structure activity comparisons / comparison with similar mixtures

- New Approach Methodologies (NAMs)
  - ´Omics studies
    - Genomics, transcriptomics, proteomics, metabolomics, epigenomics
    - High Throughput Screening (HTS) data
    - ToxCast and Tox21 assays
  - In silico systems
    - Quantitative Structure Activity Relationship (QSAR) models
    - DAVID

Exploring Use of HTS Data in Cancer Hazard Prediction

An Integrated Approach Using Publicly Available Resources for Identifying and Characterizing Chemicals for Potential Toxicity Concern: Proof-of-Concept with Chemicals that affect Cancer Pathways. Shoba Iyer, Nathalie Pham, Melanie Marty, Martha Sandy, Gina Solomon, Lauren Zeise (2019) *Toxicol Sci* doi: 10.1093/toxsci/kfz017

We developed an approach that uses current knowledge to identify cancer pathway-related *in vitro* assays from among a subset of ToxCast assays

- Characterized 236 individual assays from 3 ToxCast assay platforms (ACEA, Apredica, BioSeek)
  - Determined that 137 assays were related to cancer pathways
- Mapped the cancer pathway-related assays to individual Key Characteristics of carcinogens (KCs)
  - Only 5 KCs covered (KC #: 2, 4, 5, 6, 10)
- Analyzed ToxCast data from the cancer pathway-related assays, for all 1061 chemicals tested in Phases 1 and 2
- Used ToxPi (Toxicological Prioritization Index) software to rank the chemicals based on activity in these assays, and grouped by KC
- Used ChemoTyper to identify enriched chemotypes in the top 5% of ranked chemicals

Mapping ToxCast and Tox21 Assays to the KCs

Iyer et al 2019

Biological coverage of the subset of ToxCast assays from 3 platforms for the KCs was limited, and no assays mapped to five KCs (KC#: 1, 3, 7, 8, 9)

IARC https://monographs.iarc.fr/iarc-monographs-on-the-evaluation-of-carcinogenic-risks-to-humans-3/ & Chiu et al 2018

Mapped a more complete set of ToxCast/Tox21 assays, and found biological coverage was limited for many KCs, and no assays mapped to three KCs (KC#: 3, 7, 9)

Some key observations:

- It may not be possible to capture the biology of some of the KCs (e.g., immunosuppression, chronic inflammation) in these, or other short-term in vitro assays
- Metabolism is minimal in these assays

Recommendation:

- There is a need to "design" a set of short-term assays to specifically interrogate each of the KCs
  - Some of these assays may come from existing ToxCast or Tox21 HTS cell-free or in vitro assays.
  - Others may be newly designed HTS assays
  - Still others may be medium throughput assays, or short-term in vivo assays

Until we have closed the biological coverage gaps in our HTS, MTS and other short-term assays, we will not have sufficient confidence that we can effectively screen chemicals for carcinogenicity concern

How is California Using Mechanistic Evidence, Including HTS and ´omics Data, and KCs in Cancer Hazard Identification?

- Long-standing recognition that carcinogens can act through more than one mechanism

> Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches KZ Guyton, AD Kyle, J Aubrecht, VJ Cogliano, DA Eastomnd, M Jackson, M Keshava, MS Sandy, B Sonawane, L Zhang, MD Waters, MT Smith (2009) *Mutat Res* 681: 230-240.

- Evaluation of gene and protein expression data and ´omics data, *when available* for the chemicals under review, since 2008
- Analysis of ToxCast/Tox21 data, *when available* for the chemicals under review, since 2015
- Discussion of mechanistic evidence as it relates to Key Characteristics of Carcinogens, since 2016

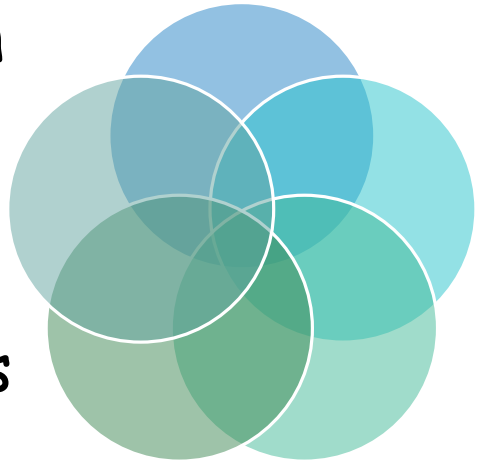
Marijuana Smoke: Multiple Possible Mechanisms of Action

Evidence on the Carcinogenicity of Marijuana Smoke
OEHHA 2009

Genotoxicity

Changes in endocrine function

Immune suppression



Changes in cell signaling

Sustained inflammation

Synthesis of mechanistic evidence organized by KC

Characteristic	Example of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (*e.g.,* epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, UDS), intercalation, gene mutations, cytogenetic changes (*e.g.,* CAs, MN)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (*e.g.,* topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (*e.g.,* DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor inactivation/activation (*e.g.,* ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Evidence on the Carcinogenicity of Gentian Violet OEHHA 2017

- Gentian violet may act via multiple mechanisms, which can be grouped according to the key characteristics of carcinogens described by Smith et al. (2016).
- These mechanisms include
  - Being electrophilic or forming electrophilic metabolites
  - Genotoxicity
  - Oxidative stress induction
  - Receptor-mediated effects

Analysis of ´omic data from rat liver following in vivo exposure to coumarin

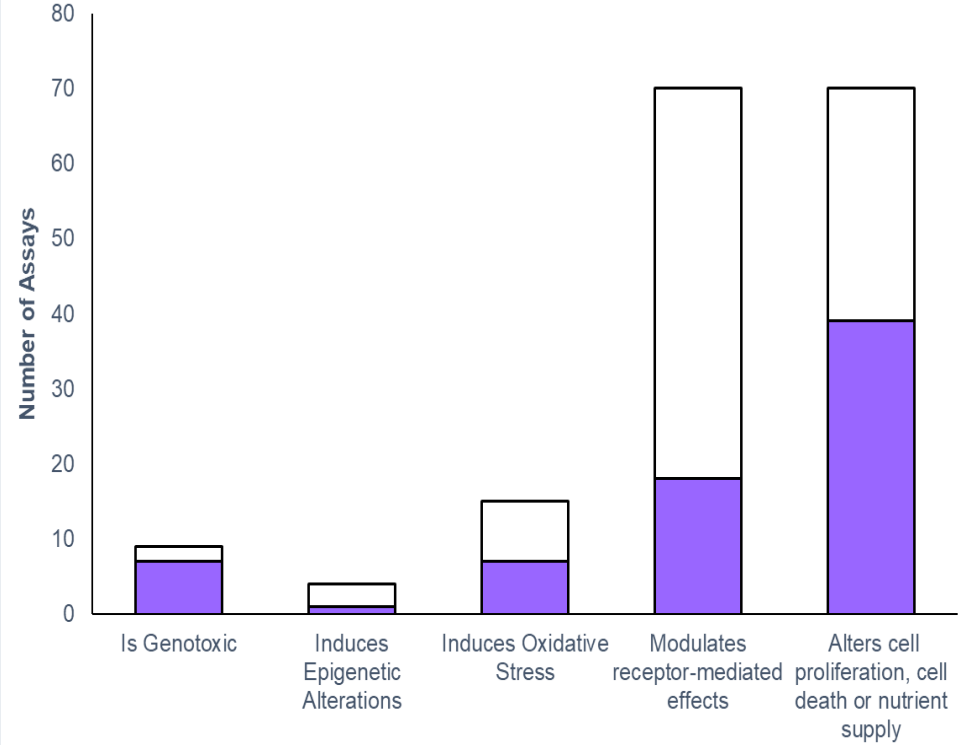
GO/Pathway	No. of genes associated with pathway/no. of genes in tested gene set	*p*-value[d]	IARC ten key characteristics of carcinogens	CTD ratio of cancer to all diseases (%)
Chemical carcinogenesis	4/69 (down[b])	< 0.01	**All 10 key characteristics**	100.00
Drug metabolism - cytochrome P450	4/69 (down)	< 0.01	**1: Electrophilic**	27.13
Metabolism of xenobiotics by cytochrome P450	4/111 (up[c])	< 0.05	**1: Electrophilic**	30.60
Secondary metabolites biosynthesis, transport, and catabolism	4/69 (down)	< 0.05	**1: Electrophilic**	36.40
Nucleotide-binding	13/111 (up)	< 0.05	**1: Electrophilic; 2: genotoxic**	30.64
Base excision repair	3/111 (up)	< 0.05	**2: Genotoxic; 3: Alters DNA repair or causes genomic instability**	40.76
DNA replication	7/111 (up)	< 0.001	**2: genotoxic; 3: genomic instability**	35.27
Glutathione metabolic process	6/111 (up)	< 0.001	**5: Induces oxidative stress**	24.78
Oxidation-reduction process	15/111 (up)	< 0.001	**5: Induces oxidative stress**	22.17
	12/69 (down)	< 0.001		
Response to oxidative stress	7/111 (up)	< 0.001	**5: Induces oxidative stress**	25.85
Antigen processing and presentation	6/111 (up)	< 0.001	**6: Induces chronic inflammation; 7: Immunosuppressive**	14.68
Calycin[a]	3/69 (down)	< 0.05	**6: Induces chronic inflammation; 8: Modulates receptor-mediated effects**	8.50
Steroid hormone biosynthesis	5/69 (down)	< 0.001	**8: Modulates receptor-mediated effects**	16.78
Cell cycle	7/111 (up)	< 0.001	**10: cell proliferation**	46.57

Evidence on the Carcinogenicity of Coumarin OEHHA 2017

- OEHHA grouped transcriptomic data into gene annotation clusters
- The KCs were applied to assist in recognizing cancer-associated pathway clusters
- Each KC was associated with at least one annotation cluster of genes with significantly altered expression in rat liver following *in vivo* coumarin treatment

Analysis of ToxCast Data on Gentian Violet

- Gentian violet was active in 273/794 ToxCast assays
- OEHHA used IARC's most recent mapping table
- 72 assays were mapped to five KCs



Use of ToxCast/Tox21 Data in OEHHA Proposition 65 Cancer Hazard Identification Since 2015

Chemical	# active assays/ total assays tested	P65 Carcinogen?
Nitrapyrin	7/403	Yes
2,3-Diaminotoluene	70/421	No
2,4-Diaminotoluene	7/392	Yes
3,4-Diaminotoluene	49/405	No
Coumarin	13/882	No
Gentian violet	273/794	Yes
N-Nitrosohexamethyl-eneimine	2/276	Yes

- For several of these chemicals, there were few active assays; may be related to limited metabolic capacity of the assays
- Data mapped to the KCs and/or cancer-related biological processes
- Overall, these data have been of limited value for cancer hazard identification

Integration of Streams of Evidence in Proposition 65 Cancer Hazard Identification

Year	Chemical	Human	Animal (# of studies)	Mechanistic Traditional	Mechanistic NAMs	P65 Carcinogen
2009	Marijuana smoke	Yes	Yes (2)	Yes	No	Yes
2010	3-Monochloropropane-1,2-diol	No	Yes (4)	Yes	No	Yes
2010	1,3-Dichloro-2-propanol	No	Yes (2)	Yes	No	Yes
2011	Tris(1,3-dichloro-2-propyl)phosphate	No	Yes (2)	Yes	No	Yes
2012	CI Disperse Yellow 3	No*	Yes (3)	Yes	No	Yes
2012	2,6-Dimethyl-N-nitrosomorpholine	No	Yes (>20)	Yes	No	Yes
2013	Diisononyl phthalate	No	Yes (6)	Yes	Yes	Yes
2013	Butyl Benzyl phthalate	Yes	Yes (2)	Yes	Yes	No
2015	Nitrapyrin	No	Yes (3)	Yes	Yes	Yes
2017	Coumarin	No	Yes (4)	Yes	Yes	No
2018	Gentian violet	No	Yes (4)	Yes	Yes	Yes
2018	N-Nitrosohexamethyleneimine	No	Yes (>20)	Yes	Yes	Yes

Moving Toward Increased Utility of NAMs in Identifying Cancer Hazards

- Current NAMs do not inform all 10 of the Key Characteristics of Carcinogens, and provide only partial coverage of the other KCs
- Need to develop new short-term screening assays and approaches that are specifically designed to interrogate each of the KCs
- Once developed, these NAMs need to be validated against evidence from animal bioassays
- Guidance is needed to further the use of mechanistic evidence from NAMs and other sources in cancer hazard prediction and identification (absence direct evidence from humans or animals)

Observations from current practice of cancer hazard identification:

- Absent evidence from cancer studies in humans, animal bioassay data continue to provide key evidence for cancer hazard identification

A Closer Look at the Utility of Animal Cancer Studies: Do all human *chemical* carcinogens, when adequately tested, induce tumors in animals?

Approach: Analyzed IARC Vol 100A-F Group 1 *chemicals*

- 57 out of 62 have *sufficient evidence* **in humans***
  - How many of the 57 have *sufficient evidence* **in animals**? 45 (79%)
  - 12 chemicals had less than *sufficient evidence* in animals, were those 12 adequately tested in animals? No
    - 4 had *no evidence* in animals
    - 2 had *inadequate evidence* in animals
    - 6 had *limited evidence* in animals

      All 6 with *limited evidence* were tested in studies with multiple design limitations, e.g., all had small group sizes (< 50 animals/group) and less-than-lifetime study durations, some as short as 20 or 24 weeks

Busulfan	Ciclosporin	Combined estrogen-progestogen contraceptives
Methyl-CCNU	Sulfur mustard	Chlornaphazine

* The other 5 Group 1 chemicals had *Limited* (1), *Inadequate* (3), or *No evidence* (1) in humans

Integration of Evidence Streams in Cancer Hazard Identification

- In the absence of human data, animal evidence continues to play a key role

Chemical

Animal evidence of carcinogenicity

Mechanistic and other relevant data

- Electrophilic or metabolized to electrophilic
- Genotoxicity
- Alteration of DNA repair, genome instability
- Epigenetic alterations
- Oxidative stress
- Induces chronic inflammation
- Immunosuppression
- Modulation of receptor-mediated effects
- Immortalization
- Alteration of cell prolif., cell death, or nutrient supply

PK and metabolism

Susceptibility factors

Structure activity comparison and QSAR

Other data

- Need better coverage of KCs by NAMs